



## Bloom in a Bottle: Water Collection and Dilution Experiment Procedure

Bloom in a bottle is a simple but versatile experiment which can be performed by students from different grade levels with a teacher's or parent's supervision. The main objective of the experiment is to highlight the pivotal role of microorganisms in the carbon cycle. Below you will find the procedure to collect raw water and perform a dilution experiment with materials easily found at home.

### Dilution Experiment Procedure

#### 1. Experimental Containers

- a. Gather 15 identical clear mason jars or water bottles. Make sure they are completely clean and all labels have been removed from clear surfaces. If you do not have mason jars, empty plastic water or soda bottles will also work. We find that 16-20oz bottles work well.
- b. Determine the volume of each container, multiply this value by 25 to determine the volume of water that needs to be collected.

#### 2. Water Collection

- a. Collect raw water from any natural freshwater source e.g., river, lake, stream, creek, pond, wetland, etc. The greener the source water the better, this means there are a lot of microbes growing in it.

#### 3. Water Preparation

- a. At home decant (pour out) 8 container volumes of raw water into a holding container such as a bucket. Set this aside in a cool, sunlit location. This will be your inoculum, so the microbes must stay alive
- b. At home decant the remaining collected water (at least 16 container volumes) into a large kitchen pot. Bring the water to a rolling boil for at least 5 min. This will kill all the microbes growing in the water and turn their biomass back into nutrients accessible to other microbes. Allow this boiled water to cool to ambient temperature overnight. The solids should also settle out. Note: if you do not have pot large enough for all your water you may boil it in batches following the same procedure as listed above.

#### 4. Experimental Setup

- a. Label 15 containers as follows
- b. Treatments (3x for each)
  - i. Negative control (all boiled water)
  - ii. 1:4 (1 part raw water, 3 parts boiled water)
  - iii. 1:2 (half raw water, half boiled water)
  - iv. 3:4 (3 parts raw water, 1 part boiled water)
  - v. Positive control (all raw water)
- b. Add the prescribed mixture of raw water and boiled water to each container
- c. Loosely cap each container to allow for gas exchange
- d. Place all containers in a sunlit area to incubate

### **Experiment Rational:**

By boiling the raw water, all organisms in the water are killed and their cells are lysed. In the process their biomass is returned to a form that other microbes can use to grow. We will then use the boiled water as growth media for the raw water.

The varying dilutions of the raw water and the boiled water test different amounts of nutrients and initial cells being added to the experiment. Treatments with more nutrients should ultimately grow to have more cells (organisms), but the growth will take longer because you're starting with fewer cells. Treatments with more cells will grow faster, but will result in a less dense final culture (not as green). The negative control should have no growth, and the positive control should have some growth (but is not expected to have the maximal density).



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